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Structure of the human oestrogen-responsive gene pS2

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ABSTRACT

The human pS2 gene, whose expression is restricted to breast cancer cells, and whose transcription is induced by oestrogen in the human breast cancer cell line MCF-7, has been cloned from both placental and MCF-7 cell DNA. The exon-intron organization has been established by electron microscopy using genomic DNA-cDNA or -mRNA hybrid duplexes and by sequencing the exons and exon-intron junctions. The overall organization within and around the pS2 gene is the same in placental and MCF-7 cell DNA and the exonic sequences are identical to those previously determined from the cDNA. The 5'-flanking region of the pS2 gene is also identical (with the exception of two base transitions) in the two tissues. Thus no gene rearrangement nor sequence modification has occurred in the pS2 gene of the malignant and polyploid MCF-7 cells. A TATA-box, a CAAT-box and a GC-rich motif are present in the 5'-flanking region of the pS2 gene, but the latter motif is unusually located between the TATA-box and the capsite. No significant homology could be detected between the 5' flanking sequences of the pS2 gene and those of other oestrogen-reponsive genes from different species.

INTRODUCTION

Cells of the human breast adenocarcinoma cell line MCF-7 (1) contain both oestrogen and progesterone receptors (2, 3), are fully transformed according to the criteria of immortalized growth and anchorage-independent proliferation in soft agar, require oestradiol for tumor formation in vivo (4) and exhibit growth-stimulation in response to oestradiol in vitro (5). Exposure of these cells to oestrogen also results in the induction of certain mRNAs and proteins and secretory growth factors (for refs and reviews, see 6, 7). MCF-7 cells appear, therefore, ideally suited for the study of the molecular basis of steroid hormone regulation and as a model for hormone-responsive tumors.

We have previously reported the isolation of a cDNA clone, termed pS2, which corresponds to an mRNA species induced by oestradiol-treatment of hormone-deprived MCF-7 cells (8). Oestradiol (but not other steroid hormones such as progestins, glucocorticoids, or androgens) induces pS2-mRNA rapidly

and directly at the transcriptional level via a mechanism which is independent of de novo synthesis of proteins (9). Thus, amongst the various oestrogen-responsive genes described in the literature to date [the most well characterized examples being the chicken egg-white protein genes (for reviews and refs see 10, 11), prolactin gene (12), avian and Xenopus laevis vitellogenin genes (13, 14 and refs therein), and the apo VLDLII gene (15)], the pS2 gene represents a unique example of a human gene whose transcription is directly controlled by oestrogen.

Detectable levels of pS2 mRNA have so far only been found in MCF- 7 cells and certain breast tumor biopsies, but not in normal breast tissue nor in any cultured human cell lines (HeLa cells, human fibroblasts) or human cells or tissues [lymphocytes, placenta, liver, endometrium (10, 16)]. Neither can pS2 mRNA be detected in the breast cancer cell line T47D, despite the fact that this line contains oestrogen receptor (10). Such observations suggest that pS2 may be of use as a marker in the identification of certain classes of oestradiol-dependent breast cancers (16). The complete sequence of the pS2 cDNA has been determined (17). Analysis of the open reading frame has indicated that the putative pS2 product corresponds to an 84 amino acid protein with a 21 or 26 amino acid signal peptide, suggesting that the mature pS2 protein may be secreted. Direct evidence supporting this possibility has been obtained recently (unpublished results from our laboratory).

The isolation and characterization of the pS2 gene from both the MCF-7 cell line and "normal" tissues is a prerequisite to investigating the molecular mechanism of oestrogen action and the interrelationship between genome organization, hormone induction and the cancerous state. We describe here Southern blot analysis of the pS2 gene in MCF-7 cells and normal tissues, and the isolation and detailed characterization of genomic clones containing the pS2 cDNA sequences, isolated from both the MCF-7 cell line and human placenta. We present the structural organization of the entire transcriptional unit of the pS2 gene determined by detailed restriction enzyme mapping, electron microscopic analysis and DNA sequencing of both 5' and 3' flanking regions, exons, and intron/exon boundaries.

MATERIALS AND METHODS

1. DNA preparation

High molecular weight human DNA was isolated from partially purified nuclei of MCF-7 cells, liver and placenta as follows :

a) Liver and Placenta (30 and 150 g., respectively), stored in liquid nitrogen, were thawed on ice, passed through the grid of a tissue press and

recovered in 5 ml of cold solution A (sucrose 250 mM, $MgCl_2$ 5.5 mM) per gram of starting tissue (pgst). This mixture was then homogenized on ice in a Dounce with 20 up and down strokes of a tightly fitting pestle and filtered through a 200 mesh nylon net. Crude nuclei were pelleted (10 min centrifugation at 1200xg), resuspended in 2 ml of cold solution A pgst (the preparation was checked by microscopy), pelleted again, resuspended in 0.75 ml of cold solution A pgst and diluted with one volume of solution B (Tris-HCl 10 mM pH 7.5, EDTA 1 mM, SDS 1% [w/v]). As lysis started, a preincubated (30 min at 37°C) solution of 10 mg/ml proteinase K (Merck) in Tris-HCl 10 mM pH 7.5, EDTA 1 mM, SDS 0.5% [w/v] and NaCl 150 mM, was added (final concentration of proteinase K : 1mg/ml) and the mixture was incubated overnight at 37°C with very gentle agitation. The solution was extracted once with an equal volume of phenol (saturated with Tris-HCl 50 mM pH 7.5, EDTA 1 mM), then with phenol- chloroform (1:1) and twice with chloroform. Sodium acetate was added to 0.3 M final concentration and the DNA precipitated with 2 volumes of ethanol. The precipitated DNA was extracted gently from the solution using a glass rod, transferred to ethanol and pelleted (30 min, 7000xg). The DNA pellet was air-dried and left to dissolve in solution C (Tris-HCl 50 mM pH 7.5, EDTA 1 mM) at a final DNA concentration of 1.5 mg/ml. (Approximative yields : 0.5 mg DNA/g. of placenta; 0.2 mg DNA/g. of liver).

b) MCF-7 cells, grown as previously described (9) in T75 Falcon flasks, were scraped into 5 ml PBS. Cells were then pelleted in a 150 ml cold preweighed Corex bottle (5 min centrifugation at 2000xg at 4°C), suspended in PBS (1/3 of the starting volume), pelleted as before and weighed, before being resuspended (1/3 of the initial volume) in solution D (sucrose 250 mM, $MgCl_2$ 5.5 mM, NaCl 2.5 mM, Tris-HCl 2.5 mM pH 7.5, Triton X100 1% [w/v], sodium deoxycholate 1% [w/v]). Cells were broken in an ice cold Potter-Elvehjem homogeneizer using a motor- driven Teflon pestle (1200 rpm) with 30 up and down strokes. Crude nuclei were pelleted by centrifugation and processed as described for liver and placenta except that the solution volumes pgst were doubled. (Yield : ≃ 350 µg DNA/Falcon flask).

2. Genomic DNA mapping

DNA, digested at a final concentration of 200 µg/ml with various restriction endonucleases (single and double digestions) according to recommendations of the suppliers, was electrophoresed in agarose gels, transferred to nitrocellulose filters or DBM-paper (18) according to Southern (19), and hybridised with nick-translated [^{32}P]-labelled probes using standard procedures (20).

3. Linear sucrose gradient DNA fractionation.

Linear sucrose gradient DNA fractionations were performed using either the SW41 or the SW27 Beckman rotor. The solutions used were as follows. 5-20% sucrose gradients : NaCl 200 mM, Tris-HCl 20 mM pH 7.5, EDTA 2mM and 5 or 20% w/v sucrose (Analar); 15-40% gradients : NaCl 1M, Tris-HCl 20 mM pH 7.5, EDTA 10 mM and 15 or 40% w/v sucrose. The maximum amount of DNA loaded per polyallomer tube was 100 µg and 350 µg for the SW41 and SW27 rotors, respectively. After centrifugation 500 µl fractions were collected from either the top or the bottom of the tubes. In the case of plasmid vector DNA and subcloned DNA fragment purification, ethidium bromide (Serva) was added to the sucrose solutions at a final concentration of 5 µg/ml. After the centrifugation the tubes were illuminated with a short wave UV lamp (254 nm) and the DNA band(s) collected from the side by puncturing the tube with a 0.5 x 16 mm needle.

DNA was precipitated from the fractions by adding 2.5 volumes of ethanol (overnight, -20°C), either directly for the 5-20% gradients or after a five-fold dilution with H_2O for the 15-40% gradients. DNA was pelleted (30 min, 18000xg), then the pellet was dried under vacuum and resuspended in Tris-HCl 10 mM pH 7.5, EDTA 1 mM at 10 to 500 µg DNA/ml.

4. Cloning of pS2 genomic DNA fragments

a) DNA fragment purification prior to cloning.

Since the pS2 gene was not present in four different human libraries which had been previously successfully screened and are supposed to be "complete" (our unpublished results; this may be due to the presence of repeated sequences within and upstream to the gene, see below), we have used the following procedure to clone the pS2 gene from MCF-7 cells and placental DNA. Human sublibraries were constructed after partial purification of pS2 gene restriction fragments. For placental DNA, 10 mg of EcoRI digested DNA were loaded on an RPC-5 chromatography column as previously described (21, 22) and the pS2 gene-containing DNA fractions were further fractionated on a 5-20% linear sucrose gradient (fractions collected from the top of the tubes). For DNA from the MCF-7 cells, 10 mg of HindIII digested DNA were fractionated through a 15-40% linear sucrose gradient and fractions collected from the bottom of the tubes; the DNA of the pooled pS2 gene-containing fractions was then digested with BglII and fractionated on a 5-20% linear sucrose gradient (the fractions were collected from the top of the tubes). The various fractions were analyzed for their pS2-gene content using the Southern transfer technique (19) and a [^{32}P]-labelled pS2 cDNA probe.

b) pS2 gene cloning.

Fractionated DNA enriched in pS2 gene sequences was cloned in the

following λ vectors : λgtWES·λc/EcoRI (23, 24) in the case of EcoRI digested placental DNA and λEMBL4/BamHI (25, 26) for the BglII digested MCF-7 cells DNA. Approximately 0.6 µg of DNA fragments were ligated to 1.2 µg of λ arms with 1 unit of T4 DNA ligase in 20 µl (4°C overnight). The ligation was monitored by 0.5% agarose gel electrophoresis. 10 µl of the ligated DNA was then packaged in vitro (20).

pS2 gene-containing phages were screened according to standard procedures using the plaque hybridisation assay (27) with [^{32}P]-labelled pS2 cDNA as probe. Minipreparation and bulk production of the recombinant phages DNA were carried out by liquid lysate methods (20, 28). After characterization of the λ recombinants, whole DNA inserts were subcloned in plasmids [pBR322 or pHP34 (29, 30)] using either the E.coli HB101 or 5K strains (31, 32). Minipreparation of recombinant plasmid DNA was performed according to (33), and bulk amounts of DNA obtained by the clear lysate method (34) were followed by cesium chloride equilibrium centrifugation.

RESULTS AND DISCUSSION

1. *The general organisation of the pS2 gene is identical in MCF-7 cells and normal human tissue*

Southern analysis of total genomic DNA isolated from a variety of cells of different origins was carried out in order to map the pS2 gene. Fig. 1A and B show Southern blots of genomic DNA from human placenta (P), liver (L), and MCF-7 cells (M), digested with the restriction enzymes HindIII, BglII, EcoRI and BamHI, and hybridised with the complete pS2 cDNA (17) as probe. In all cases, the pS2 gene is contained within a single 11.8 kb BglII fragment which itself is part of a large (≥23 kb) HindIII fragment. The 8.4 kb EcoRI and 0.7 kb BamHI (Fig. 1B) fragments give a weaker hybridisation signal relative to that of the 2.7 kb EcoRI and 3.5 kb BamHI fragments, respectively, which is correlated with the relative length of the exonic regions present in these fragments (see below). It can be seen that for a particular enzyme the same hybridisation pattern is obtained regardless of the origin of the DNA analysed, indicating that the pS2 gene has the same genomic organisation in breast cancer MCF-7 cells as in normal tissues, such as liver and placenta. The same pattern was also found in human cancer cells other than MCF-7, such as HeLa and T47-D cells for example (our unpublished observations).

Further Southern blot analysis using both the complete pS2 cDNA and purified cDNA subfragments as hybridisation probes, and both single and double restriction digest of total genomic DNA, failed to reveal any differ-

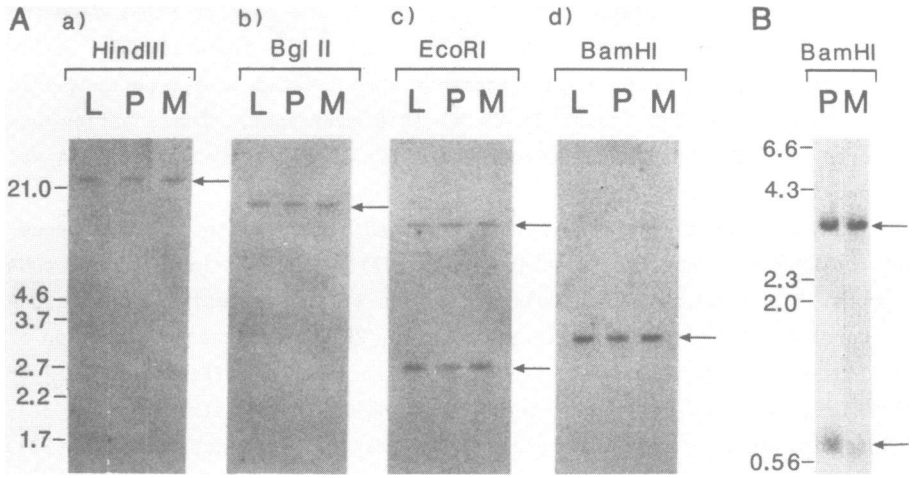

Figure 1 : *Restriction enzyme analysis of the pS2 gene region.* Total genomic DNA isolated from human liver (L), placenta (M) and the MCF-7 cell line (M), was cut with restriction enzymes as indicated, electrophoresed on 1% agarose gel, blotted onto nitrocellulose and hybridised with a [^{32}P]-labelled pS2-cDNA probe as described in Materials and Methods. Arrows point to the position of the relevant fragments. Size markers are A) fragments of EcoRI-digested Adenovirus-2 DNA and B) fragments of HindIII-digested λ phage DNA.

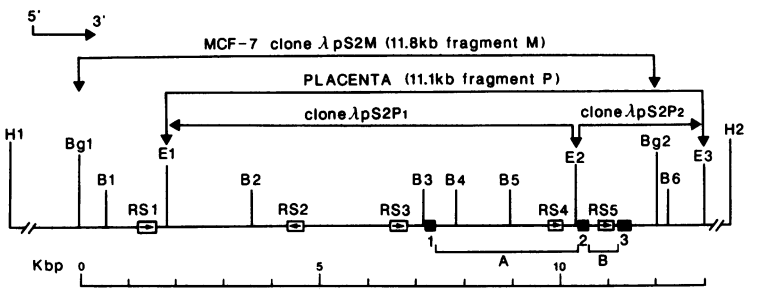

Figure 2 : *Restriction enzyme map of the pS2 gene region and structural organisation of the pS2 genomic clones isolated from DNA of MCF-7 cells and human placenta.* Restriction sites are numbered in sequence and specified as follows : H= HindIII, Bg= BglII, E= EcoRI, B= BamHI. Boxed arrowheads represent the repeated sequence elements (RS) 1-5 (see text), and indicate their relative orientation. The approximate length of the RS elements, as determined from restriction enzyme mapping and partial DNA sequencing, are 290, 250, 290, 240 and 250 bp for RS1, RS2, RS3, RS4 and RS5, respectively. The black boxes represent the exons 1, 2 and 3, which are separated by introns A and B.

ences in the structural organisation of the pS2 gene in either placenta or MCF-7 cells (data not shown). The data obtained from such analyses enabled us to establish the restriction map of the pS2 gene as shown in Fig. 2.

It is also apparent from the data shown in Fig. 1, that not only is the structural organisation of the pS2 gene conserved within liver, placenta and MCF-7 cells, but also the relative gene dosage appears to be the same, since the hybridisation signals were of similar intensity for these three DNAs. Thus, the pS2 gene has maintained its integrity in the MCF-7 cell line, despite the fact that these cells are known to be polyploid and to have undergone extensive chromosomal translocations (35). However, the occurrence of translocations encompassing the pS2 gene-containing HindIII fragment in the MCF-7 cell line cannot be ruled out.

2. Exon-intron organisation of the pS2 gene in genomic clones

Genomic banks enriched for pS2 DNA sequences of either MCF-7 cell or human placental origin, were established in λEMBL4 and λgt WES.λc, respectively (Materials and Methods). A single phage clone (λpS2M) containing an 11.8 kb BglII fragment M was isolated from the MCF-7 cell bank (Fig. 2). This fragment was cloned in pBR322 to yield pS2M. Two phage clones containing EcoRI fragments of either 8.4 kb (λpS2P1) or 2.7 kb (λpS2P2) were obtained from the human placental bank. These fragments are contiguous in the genome, and together comprise an 11.1 kb fragment P from which the E1-B6 (EcoRI-BamHI) fragment was constructed in a pBR322-based recombinant called pS2P. Results obtained from fine restriction enzyme mapping of the isolated clones were consistent with those obtained previously from Southern analysis of the endogenous pS2 gene, and did not reveal any differences between the clones from placental and MCF-7 cell origin.

The exon-intron organisation of the pS2 gene was investigated by electron microscopic analysis of hybrids formed between DNA fragments from isolated genomic clones and either the pS2 cDNA or mRNA. Fig. 3a shows a heteroduplex formed between the entire 11.1 kb genomic fragment P and a BamHI full-length cDNA fragment excised from pSVES1 (17) which illustrates that the pS2 gene contains three exons (1-3) split by two introns (A and B). The sizes of the exons and introns determined by electron microscopy are indicated in Table 1. Examination of R-loop hybrid structures resulting from hybridisation between MCF-7 poly(A) RNA and purified subcloned fragments from the 11.1 kb genomic fragment P, verified the exon-intron arrangement shown in Fig. 2. Representative examples are shown which confirm the presence of two exons within the 2.7 kb E2-E3 EcoRI fragment (Fig. 3b) and a single exon within the 0.7 kb B3-B4 BamHI fragment (Fig. 3c). Electron microscopic

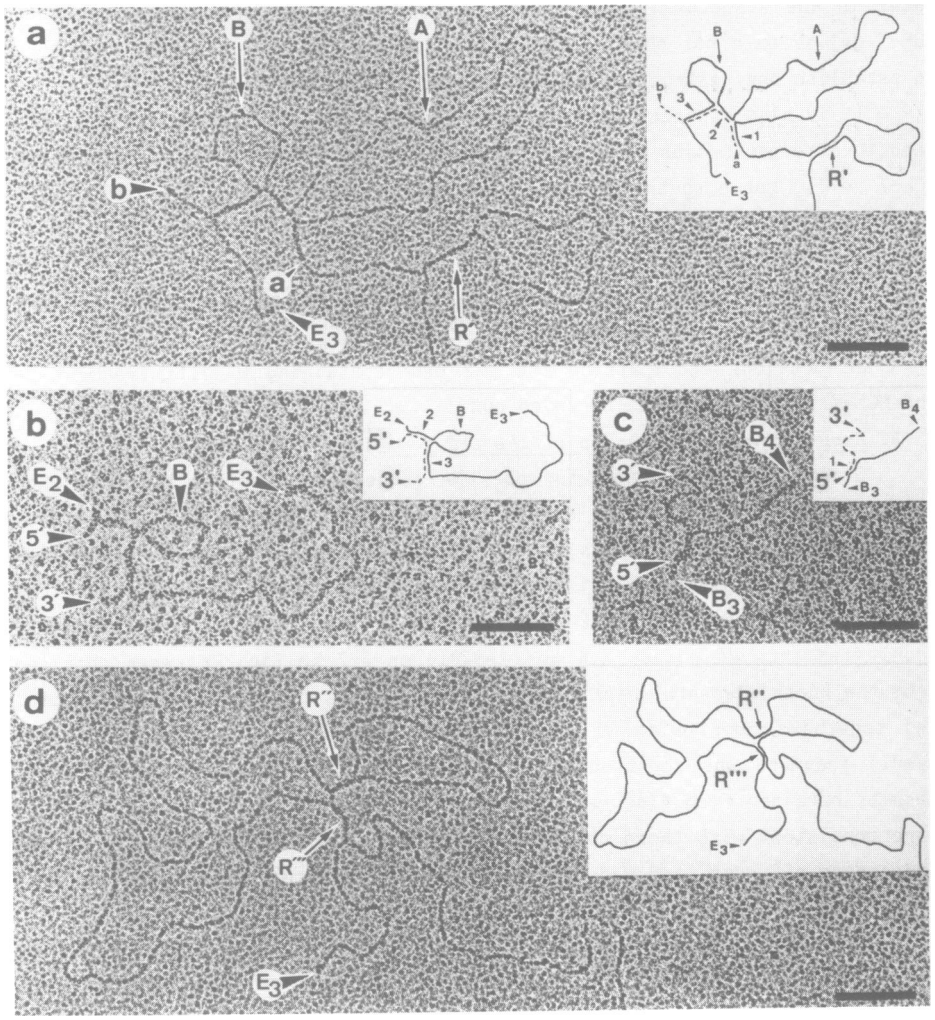

Figure 3 : *Electron microscopy of hybrids between the pS2 gene and pS2 cDNA or pS2 mRNA.* The hybrid molecules were obtained as described (41). Inserts show a schematic representation of the hybrid structures visualised in the electron microscope : continuous lines represent pS2 genomic DNA and dashed lines represent pS2 cDNA or mRNA. The bar corresponds to 0.1 μ. (a) Heteroduplex molecules between the cloned placental genomic 11.1 kb fragment P and the BamHI cDNA fragment isolated from pSVES1 (17). E3 corresponds to the EcoRI site E3 shown in Fig. 3, whereas a and b represent the 5' and 3' end, of the cDNA, respectively. The free single-stranded region b, corresponds to the poly(A) tail of the cDNA fragment. The coding sequences are visualised as double-stranded thick filaments (numbered 1-3 in the insert diagram) and the intervening sequences as single-stranded loops (A and B). The lengths of the exonic segments (1-3) and intronic loops (A and B) are

indicated in Table 1. An intramolecular duplex (R') of approximately 300 bp in length corresponding to a hybrid between the repeated sequences RS2 and RS3 is also visualised. (b) and (c) RNA-DNA hybrid molecules between pS2 mRNA and the placental pS2 genomic EcoRI fragment of 2.7 kb (b) and BamHI fragment of 0.7 kb (c). E2 and E3, B3 and B4 correspond to the respective EcoRI and BamHI sites shown in Fig. 2. 5' and 3' arrowheads correspond to the 5' and 3' ends of the pS2 mRNA, respectively. The coding sequences are visualised as double-stranded structures (exons 1-3 in the inserts), while the intron appears as a loop (B). A short free single-stranded tail corresponding to the poly(A) at the 3' end of the pS2 mRNA is observed in (b). (d) Intramolecular hybrids between repeated sequences (RS) of the E1-B6 placental genomic fragment of the pS2 gene (see Fig. 2). The hybrids between RS2 and RS3 (R") and RS2 and RS5 (R '") are visualised as double-stranded thick filaments. The lengths of the intramolecular hybrids R" and R '" are approximately 90 and 150 bp, respectively.

analysis of hybrids involving the genomic fragment M gave similar results (data not shown).

3. Sequencing of the pS2 gene and flanking regions

The precise locations of the introns were delineated by sequencing through the exon-intron junctions of the genomic fragments M and P, and comparison with the sequence of the previously published full length cDNA (17). The exon and adjacent intron sequences of the pS2 genomic clone from both human placenta (P) and MCF-7 cells (M) are identical (Fig. 4 and data not shown) and the exon-intron junctions followed the GT/AG rule (36). The transcribed region of the pS2 gene consists of three exons (1-3) of 125, 153 and 212 bps respectively, interrupted by 2 introns A and B of approximately 3.1 kb and 0.77 kb (Table 1). These lengths are in good agreement with those

Table 1 : *Lengths of pS2 gene introns and exons (in base pairs) as determined by electron microscopy (i) and restriction enzyme mapping and DNA sequencing (ii).*

		Length	
Exon	Intron	(i)	(ii)
1		118 ± 23	125
	A	3069 ± 80	≃ 3100
2		162 ± 16	153
	B	799 ± 31	≃ 770
3		206 ± 12	212

Figure 4 : *The nucleotide sequence of the pS2 gene.* Sequences were determined from both clone pS2M and clone pS2P using mainly the dideoxy-nucleotide chain termination method (42) and M13 vectors (43, 44), and in few cases the chemical degradation method (45). The sequence obtained from the M clone is shown. The cap site (+1) is indicated as well as the exon/intron boundaries (open triangles). The two base differences found (up to -328) between the MCF-7 cell clone M and the placental clone P are indicated below the sequence of the M clone. The 5' flanking region has been numbered negatively (a dot is present every 10 nucleotides). Three sequences are underlined : the CAAT-like box (dashed line), the TATA box and the ATTAAA polyadenylation signal (full line). Closed circles indicate the possible polyadenylation sites. The deduced pS2 protein sequence is given along with the exons.

determined by electron microscopy. The sequence of the three exons was identical to that of the full-length cDNA as previously determined (17).

The nucleotide sequence of the 5'-flanking region of the pS2 gene was determined to -698 in the case of the MCF-7 genomic fragment M, and to -328 in the case of the placental genomic fragment P (Fig. 4). Comparison of the P and M sequences reveals only two base changes at -304 and -185, both involving A / G transitions.

A "TATA" box sequence 5'- TATAAAA-3' is located 29 to 23 bp upstream from the start site for transcription as previously determined by primer extension (17). A sequence 5'-GGCAAAATGT-3', homologous to the "CAAT" element [consensus sequence 5'-GGPyCAATCT-3' (36,37)], commonly found 60 to 100 bps upstream from the transcriptional start site in many RNA polymerase class B (II) promoters, is present at -72 to -64 in the pS2 gene promoter. A second "CAAT"-like sequence, 5'-GGCTAATTT-3' is located at positions -639 to -631. Furthermore, the GC-rich motif 5'-GGGCGG-3' which interacts with the transcription factor Sp1 and is involved in the efficiency of transcription from a variety of unrelated cellular and viral promoters (38), is also present in the pS2 gene 5' flanking region, at -12 to -7. It will be interesting to investigate its possible role in the pS2 promoter, since up to now functional copies of this motif have all been found upstream from the "TATA" box element. In this respect it may be relevant that the bases adjacent to the pS2 gene GC-rich motif do not correspond to those present in the consensus motif 5'-$\frac{G}{T}$GGGCGG$\frac{GGC}{AAT}$-3' (see ref. 38).

4. Alu family sequences within and upstream from the pS2 gene

Five repeated sequence (RS) elements present within the cloned pS2 gene region were initially detected and mapped by Southern blot analysis of the pS2 genomic fragments M and P, employing total genomic DNA of either human placenta or MCF-7 cell origin as nick-translated probe (data not shown). The organisation of the RS elements in the genomic fragment P of placental origin, was found to be identical to that in the MCF-7 genomic fragment M. Three of these sequences (RS1, 2 and 3) are present in the 5' non-coding region of the pS2 gene, the remaining two (RS4 and 5) being located in intron A and B, respectively (Fig. 2).

Cross-hybridisation studies were performed in order to determine the possible homology between individual RS elements. A Southern blot analysis was carried out with subcloned restriction fragments harbouring single RS elements as nick-translated probes. Under stringent hybridisation conditions, all five RS elements cross-hybridised (data not shown).

DNA sequencing (data not shown) of the RS elements of the pS2 genomic fragment M revealed that all five are members of the Alu family of repeated sequences and are between 70 to 85% homologous to the Alu consensus sequence (39). The orientation of RS2 is opposite to that of the other RS elements; this arrangement results in the formation of stem-loop structures by intramolecular hybridisation of denatured pS2 DNA which can be observed by electron microscopy. For example, Fig. 3a shows an intramolecular duplex, R' (≃ 320 bp in length), involving a hybrid between RS2 and RS3, and Fig. 3d illustrates a more complex interaction involving a hybrid, R" (≃ 90 bp in length), between RS2 and RS3, and a hybrid R'" (≃ 150 bp length) between RS2 and RS5.

5. A search for sequence homology with other oestrogen-controlled genes.

The pS2 gene is the only example to date of a human gene whose expression is controlled directly at the transcriptional level by oestrogen (9). Other oestrogen-responsive genes which have been cloned and characterised, are from non-mammalian systems such as avian and amphibian liver, and avian oviduct (see Introduction). Computer assisted searches were carried out to look for homologies between the 5'-flanking sequence of the pS2 gene and those of these genes [particularly with their putative oestrogen responsive elements (13 and 14, 40 and refs therein)], in the hope that any regions conserved between oestrogen-responsive genes from these different species might be functionally involved in mediating the oestrogen response. Several regions of homology (approximately 80% homology over 14 to 20 bp) were detected between the pS2 gene and the other oestrogen-responsive genes. However they were not in similar locations with respect to the start sites and were also found in 5'-flanking regions of genes whose expression is not regulated by oestrogens. Thus, the physiological significance of these regions, if any, is unknown at present time.

Identification of those structural elements which are involved in mediating the oestrogen-response is dependent upon functional analysis of the pS2 gene. We have recently found that 1.1 kb of pS2 gene 5'-flanking region is sufficient to confer the oestrogen response to the pS2 promoter (M. Berry, unpublished results), clearly demonstrating that oestrogen induces the expression of the pS2 gene by increasing the rate of transcription from the promoter. Further experiments are underway to identify the corresponding oestrogen-responsive element(s) at the base level.

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REFERENCES

1. Soule, H.D., Vasquez, J., Long, A., Albert, S. and Brennan, M.J. (1973) J. Natl. Cancer Inst. 51, 1409-1416.
2. Brooks, S.L., Locke, E.R. and Soule, H.D. (1973) J. Mol. Biol. Chem. 248, 6251-6253.
3. Horwitz, K.B. and McGuire, W.L. (1978) J. Biol. Chem. 253, 2223-2228
4. Shafie, S.M. (1980) Science 209, 701-702.
5. Lippmann, M.E. and Bolan, G. (1975) Nature 256, 592-573.
6. Adams, O.J., Edwards, P.D. and McGuire, W.L. (1983) in Regulation of Gene Expression by Hormones, Millems, W.K., ed. (Plenum Press), pp 1-25.
7. Westley, B., May, F.E.B., Brown, A.M.C., Krust, A., Chambon, P., Lippman, M.E. and Rochefort, H. (1984) J. Biol. Chem. 84, 10030- 10035.
8. Masiakowski, P., Breathnach, R., Bloch, J., Gannon, F., Krust, A. and Chambon, P. (1982) Nucleic Acids Res. 10, 7895-7903.
9. Brown, A.M.C., Jeltsch, J.M., Roberts, M. and Chambon, P. (1984) Proc. Natl. Acad. Sci. USA 81, 6344-6348.
10. Chambon, P., Dierich, A., Gaub, M.P., Jakowlev, S., Jongstra, J., Krust, A., LePennec, J.P., Oudet, . and Reudelhuber, T.L. (1984) in Recent Progress in Hormone Res. 40, 1-42.
11. Palmiter, R.D., Mulvihill, E.R., Shepherd, J.H. and McKnight, G.S. (1981) J. Biol. Chem. 256, 7910-7916.
12. Maurer, R.A. (1985) DNA 4, 1-9.
13. Saluz, H.P., Jiricny, J. and Jost, J.P. (1986) Proc. Natl. Acad. Sci. USA 83, 7167-7171.
14. Seiler-Tuyns, A., Walker, P., Martinez, E., Mérillat, A-M., Givel, F. and Wahli, W. (1986). Nucleic Acids Res. 14, 8755-8770.
15. Chan, L. (1983) Ann. Rev. Physiol. 45, 615-623.
16. Gairard, B., Krust, A., Rio, M.C., Koehl, C., Bellocq, J.P., Chambon, P. and Renaud, R. In "Endocrinology of the Breast : Basic and Clinical Aspects", Annals of the New York Academy of Sciences, vol. 464, A. Angeli, H.L. Bradlow and L. Dogliotti (Eds), The New York Academy of Sciences (1986), pp. 443-447.
17. Jakowlev, S.B., Breathnach, R., Jeltsch, J-M., Masiakowski, P. and Chambon, P. (1984) Nucleic Acids Res. 12, 2861-2878.

18. Alwine, J.C., Kemp, D.J., Parker, B.A., Reiser, J., Renart, J., Stark, G.R. and Wahl, G.M. (1979) Methods in Enzymology 68, 220-242.
19. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
20. Maniatis, T., Fritsch, E.F. and Sambrook, T. (1982) Molecular Cloning (Cold Spring Harbor, New York : Cold Spring Harbor Laboratory).
21. Mandel, J.L., Breathnach, R., Gerlinger, P., LeMeur, M., Gannon, F. and Chambon, P. (1978) Cell 14, 641-653.
22. Pearson, R.P., Weiss, J.S. and Kelmas, A.D. (1971) Biochim. Biophys. Acta 288, 770-774.
23. Enquist, L., Timeier, D., Leder, P., Weisberg, R. and Sternberg, N. (1976) Nature 259, 596-598.
24. Murray, N.E., Brammar, W.J. and Murray, K. (1976) Molec. Gen. Genet. 150, 53-61.
25. Karn, J., Brenner, S., Barnett, L. and Cesareni, G. (1980) Proc. Natl. Acad. Sci. USA 77, 5172-5176.
26. Frischauf, A.M., Lehrach, H., Poustka, A. and Murray, N. (1983) J. Mol. Biol. 170, 827-842.
27. Benton, W. and Davis, R. (1977) Science 196, 180-182.
28. Davis, R.W., Bostein, D. and Roth, T.R. (1980) Advanced Bacterial Genetics (Cold Spring Harbor Laboratory, New York).
29. Bolivar, F., Rodriguez, R.L., Greene, P.J., Betlach, M.C., Heyneker, H.L. and Boyer, H.W. (1977) Gene 2, 95-113.
30. Prentki, P. and Krisch, H.M. (1982) Gene 17, 189-196.
31. Boyer, H.W. and Roulland-Dussoix, D. (1969) J. Mol. Biol. 41, 459-472.
32. Hubacek, J. and Glover, S.N. (1970) J. Mol. Biol. 50, 111-127.
33. Holmes, D. and Quigley, M. (1981) Anal. Biochem. 114, 193-197.
34. Clewell, D.B. and Helinksi, D.R. (1969) Proc. Natl. Acad. Sci. USA 62, 1159-1166.
35. Cowan, K.H., Goldsmith, M.E., Levine, R.M., Aitken, S.C., Douglass, E., Clendeninn, N., Nienhuis, A.W. and Lippmann, M.E. (1982) J. Biol. Chem. 257, 15079-15082.
36. Breathnach, R. and Chambon, P. (1981). Ann. Rev. Biochem. 50, 349-383.
37. Benoist, C., O'Hare, K., Breathnach, R. and Chambon, P. (1980) Nucleic Acids Res. 8, 127-142.
38. Jones, K.A. and Tjian, R. (1985) Nature 317, 179-182.
39. Schmid, C.W. and Jelinek, W.R. (19..) Science 216, 1065-1070.
40. Klein-Hitpass, L., Schorpp, M., Wagner, U., Ryffel, G.U. (1986) Cell, 46, 1053-1061.
41. Oudet, P. and Schatz, C. (1985) Nucleic Acid Hybridization : A Practical Approach. Hames, B.D. and Higgins, S.J. (Eds.), IRL Press, pp. 161-178.
42. Sanger, F., Nicklen, J. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
43. Messing, J., Crea, R. and Seeburg, P.H. (1981) Nucleic Acids Res. 9, 309-321.
44. Norrander, J., Kempe, T. and Messing, J. (1983) Gene 26, 101-106.
45. Maxam, A.M. and Gilbert, W. (1980) Methods in Enzymology 65, 499-560.